Assessment of Bioavailable Nickel in Soil Using Nickel-Depleted Urease

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Introduction

- Nickel (Ni) is a heavy metal that generally exists as an ion in the environment at very low levels (eastern U.S. soils background level: 0.5–25 mg/kg).
- It is considered a micronutrient for some crops, but may become toxic to plants, animals and humans if normal levels are exceeded.
- Human exposure pathways include air, water, food, cigarettes and skin contact.

Introduction

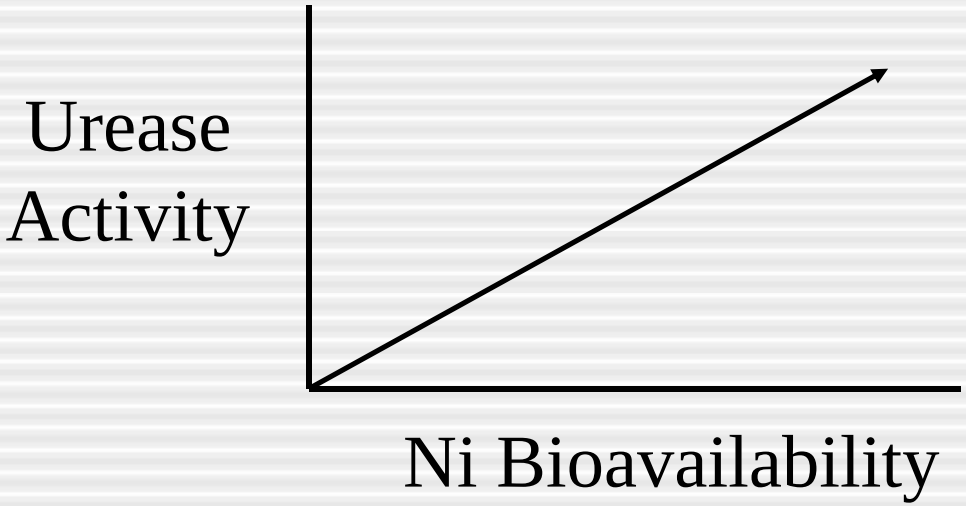
- The bioavailable concentration of Ni in soil must be accurately measured to properly assess its potential risk to plants, animals, and humans.
- Bioassays can be the ideal solution for determination of bioavailable heavy metal content of environmental samples (direct measurement of biological response, relatively inexpensive, rapid, sensitive and easy in sample and data handling).

Introduction

- Complex formation of metal ions at binding and active sites are necessary for the catalytic activity of some enzymes.
- Presumably, if these metals are limiting, the activity of the corresponding enzymes is also reduced.
- Urease is a common soil enzyme that may require up to 12 atoms of Ni for activity.

Introduction

If Ni bioavailability is limiting urease activity, we should be able to restore an increased level of urease activity by adding to the soil some bioavailable Ni.



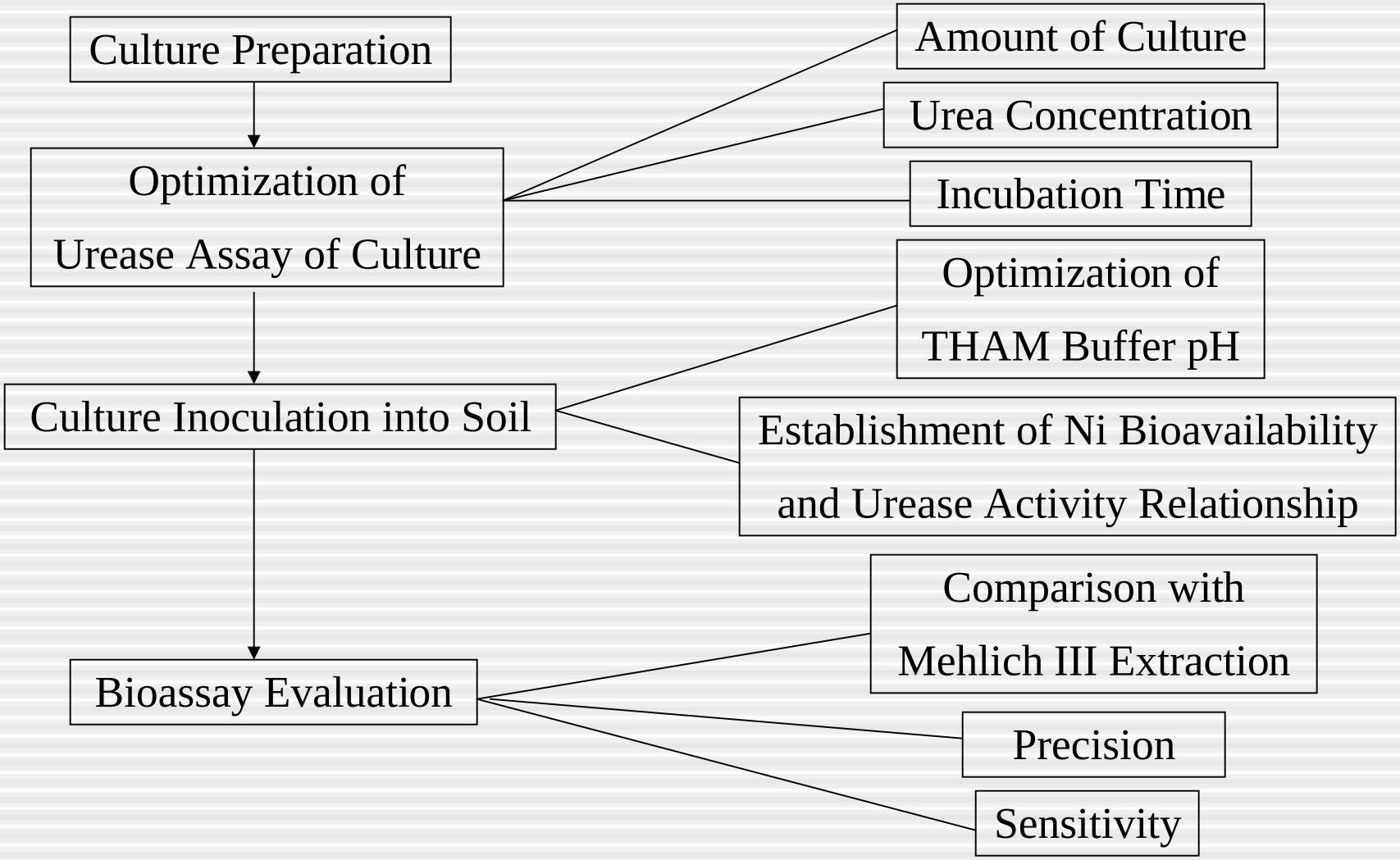
Hypothesis

- Soil enzymes that require specific metal ions for activity can be used to determine the bioavailability of those metals in soil.
- Specifically, Ni is required for urease activity and thus urease can be used to determine the bioavailability of Ni in soil.

Objectives

1) To develop microbial cultures possessing the Ni deficient urease.
2) To develop a urease bioassay to measure Ni bioavailability in soil.
3) To test the sensitivity, precision and ease of use of the urease bioassay method to evaluate Ni bioavailability in natural soils.

Materials & Methods



Materials & Methods Standard Procedure

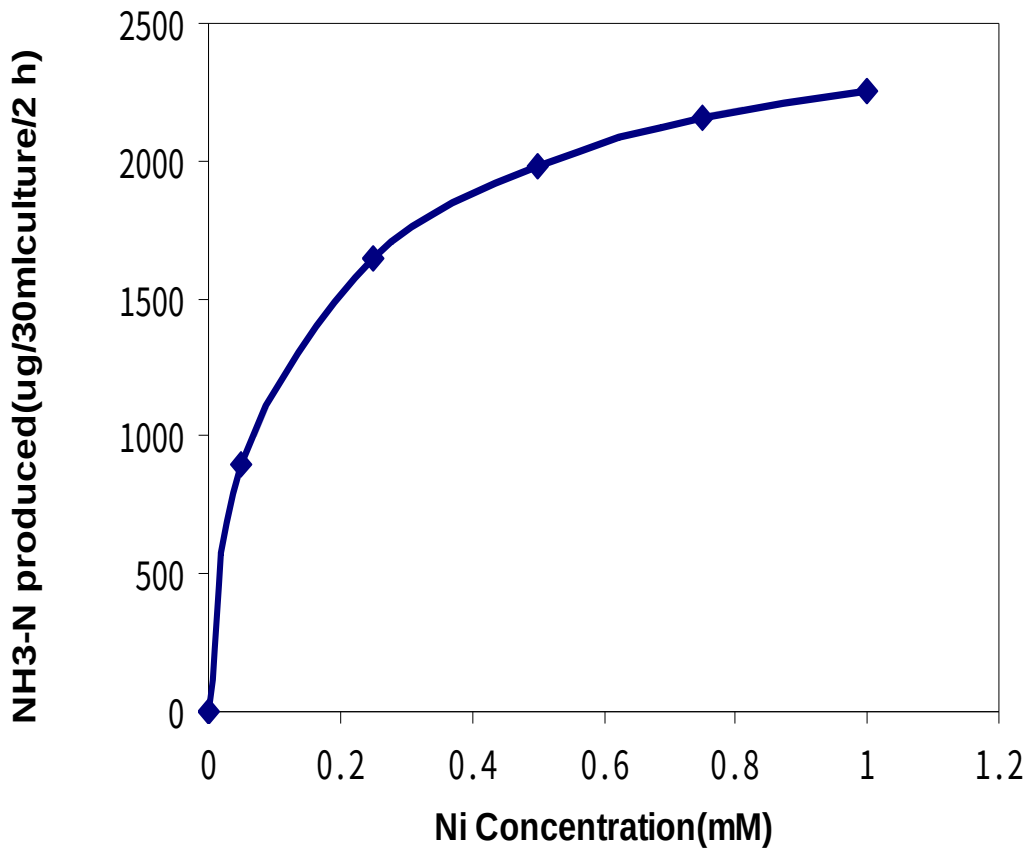
- Thirty ml of culture was inoculated into 250 mg test soil that was composed of acid washed Spinks sandy soil as a carrier soil and the soil with unknown Ni bioavailability at a ratio of 95:5.
- Culture cells were equilibrated with bioavailable Ni in soil for four hours.

Materials & Methods
Standard Procedure

- Urease activity was measured through steam distillation at a urea concentration of 0.08 M, a THAM buffer pH of 7.5, and an incubation time of 2 hours at 37℃.

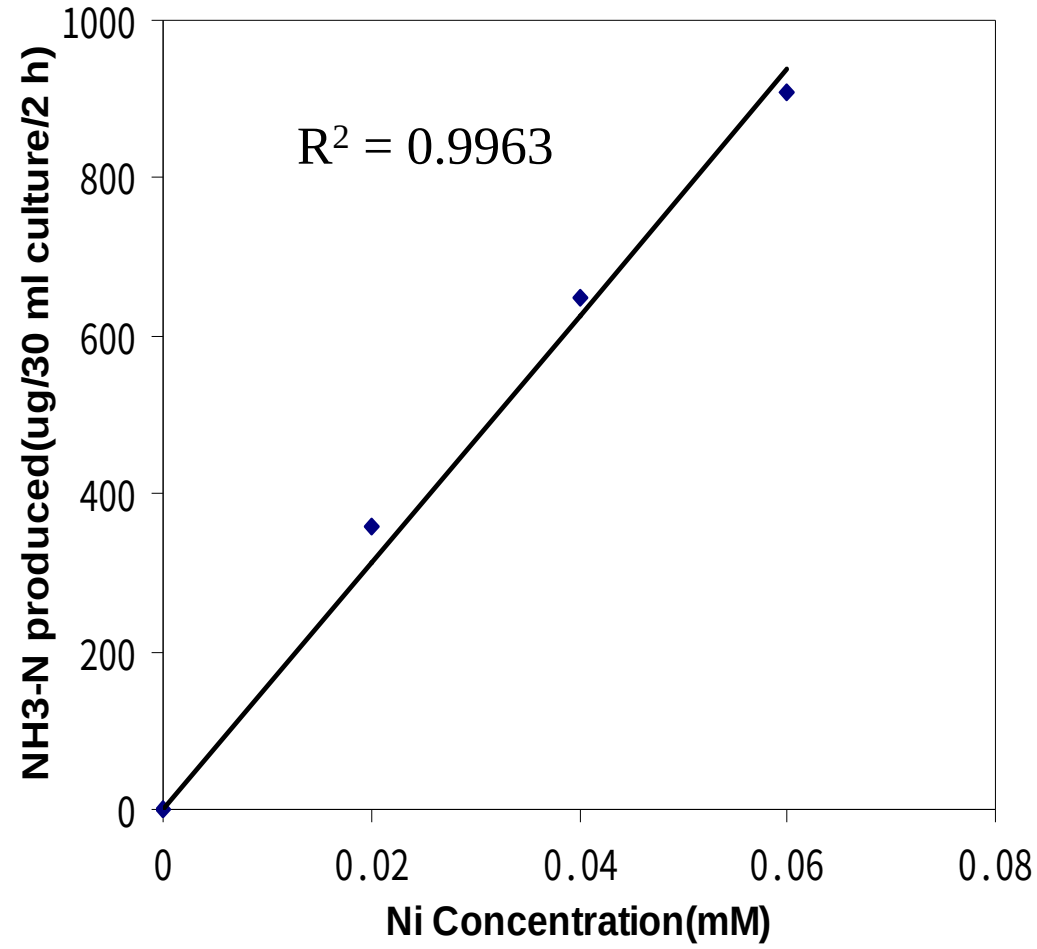
$$NH_2CONH_2 + H_2O \xrightarrow{\text{Ni + urease}} CO_2 + 2NH_3$$

Results and Discussion



Urease response to Ni concentrations from 0 to 1mM for assay of microbial cell culture under the standard procedure.

Results and Discussion



Urease response to Ni concentrations from 0 to 0.06 mM for assay of culture under the standard procedure.

Results and Discussion

Ni Bioavailability = Urease Activity / k / f

Ni Bioavailability has a unit of mM;

Urease Activity has a unit of μg/30 ml culture/2 h;

k is the slope of the regression line, which is equal to 15646 μg/30 ml culture/2 h/mM;

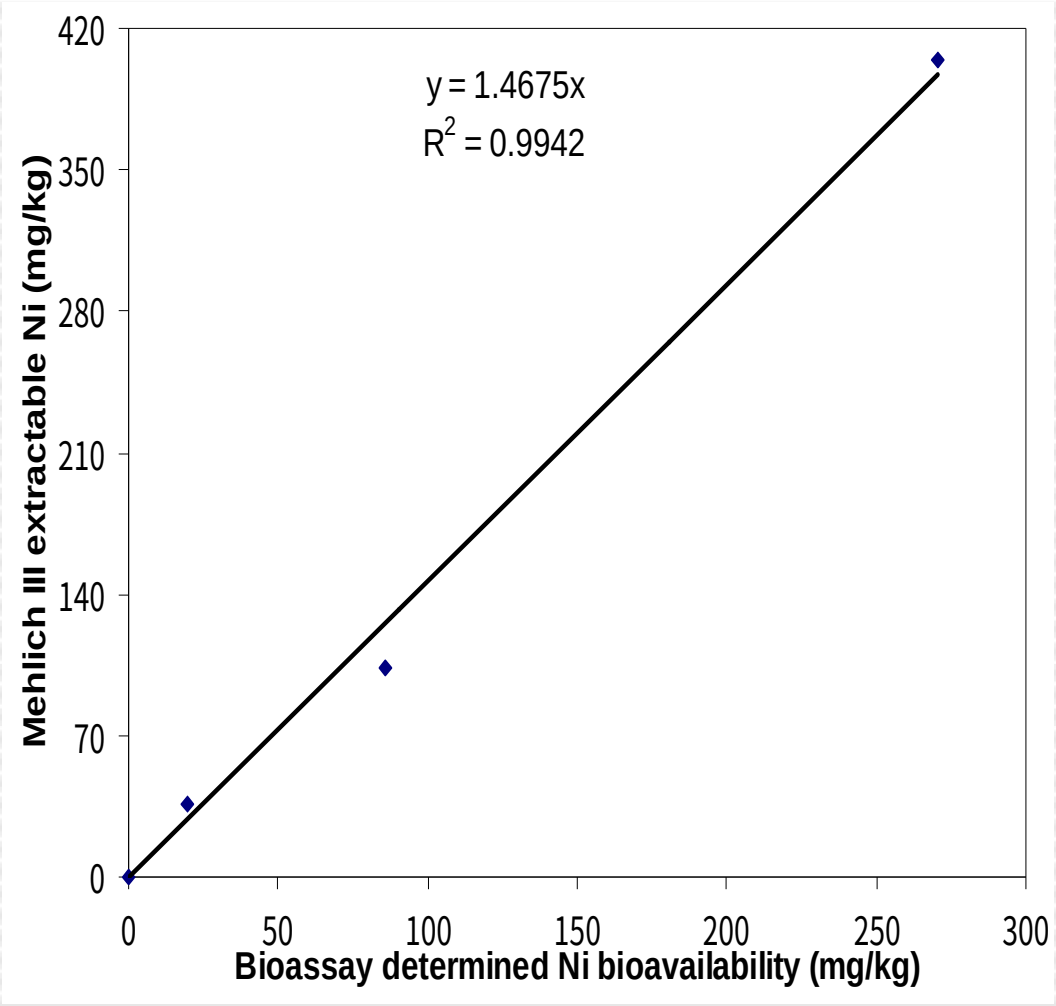
f is the percentage of unknown soil in the test soil.

Results and Discussion

Ni bioavailability in artificially Ni contaminated Wooster soils.

Soil	Ni added	Ni Bioavailability	
		Bioassay	Mehlich III
	mg/kg	mg/kg	mg/kg
Wooster (pH 7.0)	50	13.9	25.9
	200	59.7	96.1
	1000	259	352
Wooster (pH 4.8)	50	19.9	36.4
	200	85.6	103
	1000	271	404

Results and Discussion



Correlation between bioassay determined bioavailable Ni and Mehlich III extractable Ni in Wooster soil with pH 4.8.

Results and Discussion

Precision of the bioassay method for artificially Ni contaminated (200 ppm) Wooster soils.

	Ni Bioavailability			
Soil	Range	Mean	SD	CV
	----------mg/kg----------			
Wooster soil (pH 7.0)	51.8-63.7	58.5	4.30	7.35
Wooster soil (pH 4.8)	73.7-97.6	85.6	8.82	10.3

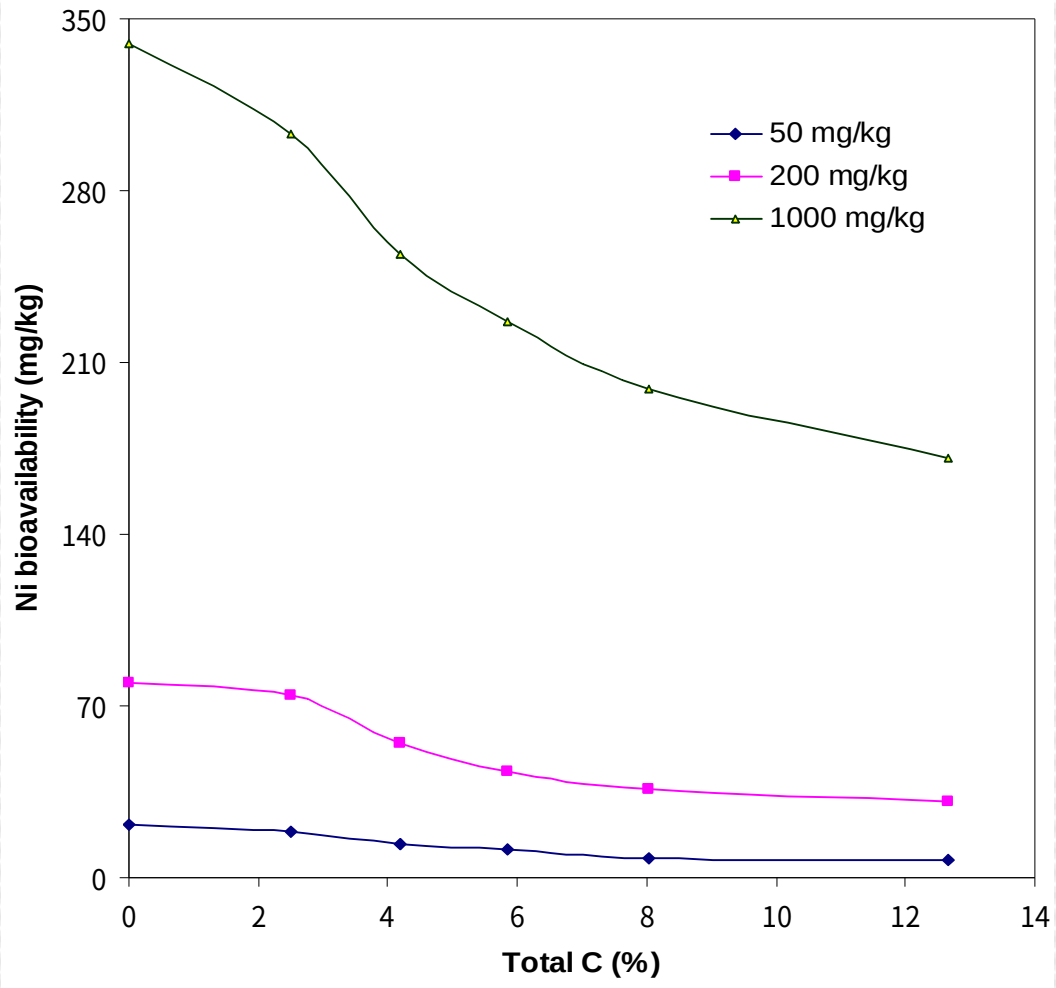
Results and Discussion

The detection limit of the bioassay method was 0.282 μM bioavailable Ni on culture base or 1.99 mg/kg bioavailable Ni on soil base. Higher sensitivity can be achieved by optimizing the urease bioassay.

Results and Discussion

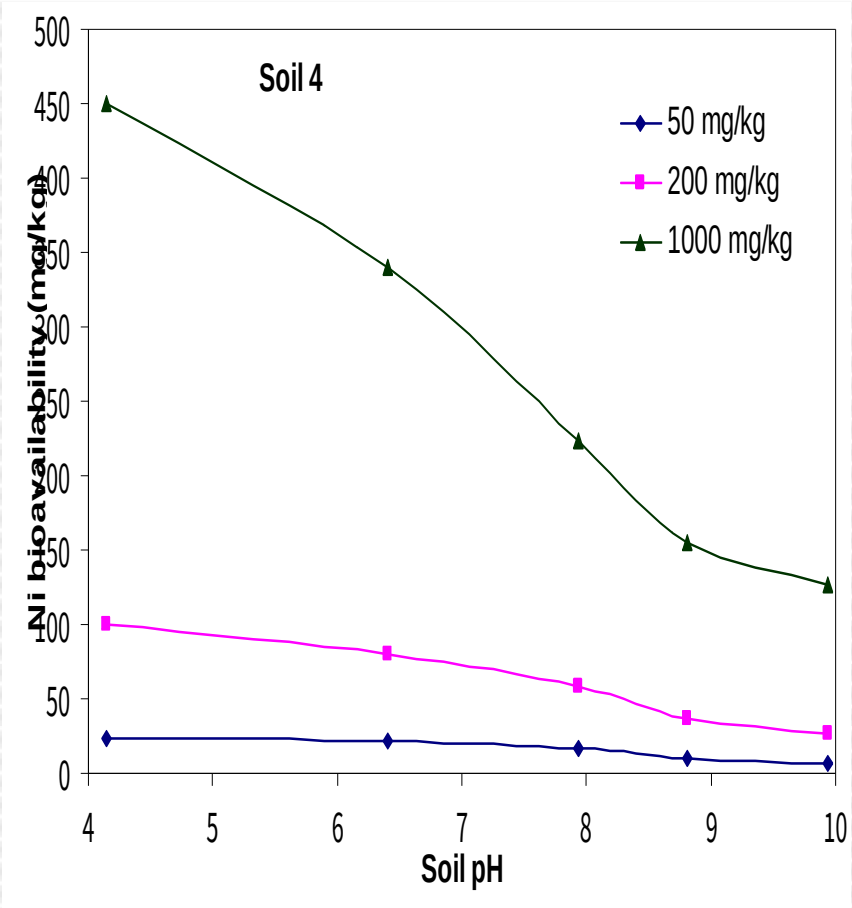
- The urease bioassay was applied to twenty soil samples with different soil pH and total C content. The natural level of Ni bioavailability measured ranged from 19.9 mg/kg to 68.3 mg/kg, showing that the urease bioassay can be applied to soils with different properties.
- The effects of soil pH and soil organic matter content on Ni bioavailability were evaluated through urease bioassay.

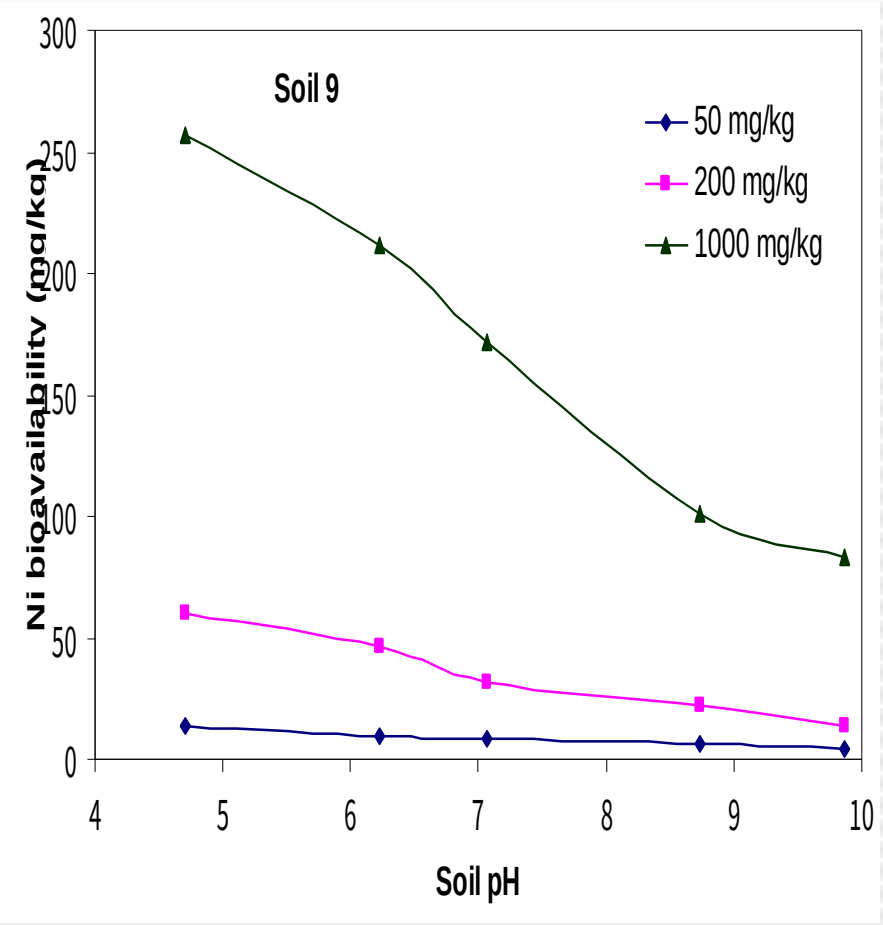
Results and Discussion



Effect of soil total (organic) C content on Ni bioavailability measured by urease bioassay.

Results and Discussion





Effect of soil pH on Ni bioavailability measured by urease bioassay

Conclusion

- Urease can be used to determine the bioavailability of Ni in soil.
- The urease bioassay was able to detect as little as 1.99 mg/kg of bioavailable Ni in soil or 0.282 μM of bioavailable Ni in culture.
- The coefficient of variation for the urease bioassay was around 10%, indicating good precision.

Conclusion

- A significant correlation (R^2=0.9945) was observed between the data from bioassay method and Mehlich III extraction, showing that the bioassay method provides an indication of the bioavailable Ni content in the soil samples similar to that of chemical extraction.

Conclusion

- The urease bioassay can be applied to soils with different properties.
- Nickel bioavailability decreased with an increase in total C content and soil pH.
- Our bioassay method is accurate, sensitive, simple, and can be used in most laboratories.

Thank You